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Research article

Community diversity shapes persisting soil legacy effects of individual plant species on subsequent plant performance

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Positive plant diversity–productivity relationships have partly been attributed to decreasing abundances of belowground specialist plant antagonists with increasing plant diversity, resulting in less negative plant–soil feedbacks (PSFs) on establishing conspecific plant individuals. Yet, generalist plant antagonists also contribute to negative PSFs among conspecific and heterospecific plants. Possibly, contributions of generalist plant antagonists to PSF outcomes may become more important in high-diversity communities due to diversity-driven specialist plant–antagonist dilution, but this has remained untested. We set up a three-phase pot experiment to examine how plant diversity affects the differences between conspecific and heterospecific PSFs. In the first (‘pre-conditioning’) phase, we conditioned soils with communities of one, two, four or eight species. By mixing soils within each diversity level, we created soil mixtures with legacies of all eight species, which only differed in the diversity level at which the plants conditioned the soils. We then established monocultures of all eight species in each mixture (‘conditioning phase’) and finally examined feedback responses of all individual species (‘feedback phase’). We tested the hypothesis that compared to heterospecific PSFs, conspecific PSFs are most negative in soil mixtures with a legacy of the lowest plant diversity, but become less negative in soil mixtures with legacies of increasing plant diversity. Diversity legacy and pre-conditioning-phase conspecific biomass positively or negatively affected plant performance in the feedback phase, depending on species identity. Plants experienced more negative conspecific than heterospecific feedback, but this effect did not depend on soil legacies established in the pre-conditioning phase. Our study shows that diverse plant communities leave persistent soil legacies that differ from combined legacies of lower-diversity communities, indicating that plant–soil interactions of individual plants are affected by the community context. However, our study does not provide evidence that community diversity predictably affects differences between conspecific and heterospecific PSF interactions.

Keywords: dilution effect, diversity–productivity relationship, plant–soil feedback, plant–soil interactions



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Introduction

In recent decades, research addressing the consequences of plant diversity loss for ecosystem functioning has shown that grassland plant community productivity typically increases with plant species richness (Tilman et al. 1996, 1997, van Ruijven and Berendse 2005). Such positive biodiversity–productivity relationships have in part been attributed to positive impacts of plant trait variation on community productivity, underlain by niche complementarity in diverse plant communities (Cardinale et al. 2007, Zuppinger-Dingley et al. 2014, Barry et al. 2019). Reduced negative impacts of plant-antagonistic organisms and increased impacts of plant-beneficial organisms through shifts in relative abundances of both groups may further contribute to the improved productivity of diverse grassland communities (Maron et al. 2011, Wang et al. 2019, Thakur et al. 2021, Zhang et al. 2024). Consequently, net impacts of soil communities on plant–community biomass (i.e. ‘soil feedback effects’) have been shown to become less negative with increasing plant community diversity (Schnitzer et al. 2011, Kulmatiski et al. 2012, Maron et al. 2016, Wang et al. 2019, Thakur et al. 2021). Possibly, such plant-diversity-driven changes in soil communities may also alter variation in soil-community-mediated interactions between individuals of the same and individuals of different species. However, how soil community development under plant communities of different levels of diversity affects subsequent soil–feedback interactions among conspecific and heterospecific plant individuals remains poorly understood.

Studies on plant–soil feedbacks (PSFs) in natural and agricultural grassland ecosystems have shown that plants often exhibit a poor performance when growing in soil previously conditioned by conspecific individuals compared to when growing in soil conditioned by heterospecific individuals (Kulmatiski et al. 2008, van der Putten et al. 2013, Semchenko et al. 2022). These results suggest that host-specialized belowground plant antagonists are key drivers of PSFs. Indeed, there are examples of important host-specialized pathogens and root herbivores that drive negative soil feedbacks on conspecific individuals and thereby contribute to succession of natural vegetation or – in agricultural systems – the need for crop rotation (van der Putten et al. 1993, Mariotte et al. 2018). However, many plant-antagonistic and plant-beneficial soil biota exhibit broad host ranges (Semchenko et al. 2018). While potentially affecting many different plant species, host generalists may still contribute to PSF variation among species, as their impacts on plant performance strongly depend on plant investments in belowground defences (Wilschut et al. 2019, 2023). For example, soil communities accumulated by plants that invest few resources into defence mechanisms may not only strongly negatively affect conspecific individuals, but also individuals of other weakly defended plant species (Wilschut et al. 2023). While this important role of host generalists as drivers of PSFs has recently been recognized (Semchenko et al. 2022), the relative contributions of host specialists and host

generalists to conspecific and heterospecific PSF outcomes remain unknown. Because the presence and abundance of host specialists depend on host abundance, their relative contributions to PSFs are likely strongest in low-diversity communities and may decrease with increasing plant diversity (Maron et al. 2016, Mommer et al. 2018). In contrast, relative abundances of host generalist plant antagonists may be expected to be less affected by increasing plant diversity, although positive effects of plant diversity on disease-suppressive soil biota may contribute to reductions of overall plant-antagonist densities (Latz et al. 2012). As such, differences between (specialist-driven) negative PSF effects on conspecific individuals and (generalist-driven) negative PSF effects on heterospecific plant individuals may decrease with increasing diversity, but this remains untested.

Differences in PSF effects on conspecific and heterospecific plant individuals are in the first place determined by the ability of plant-associated biota of one plant species to negatively or positively affect the performance of another plant species. As phylogenetically closely related plant species typically exhibit similar traits important for plant interactions with plant antagonists and plant mutualists (Gilbert and Parker 2016), they are more likely to share specialist pathogens and mutualists and to accumulate overall more similar communities of soil biota than distantly related plant species (Gilbert and Webb 2007, Lugo et al. 2015, Wang and Sugiyama 2020). Consequently, differences between soil feedback responses in conspecific-conditioned and heterospecific-conditioned soils are likely smaller for pairs of closely related species than for pairs of distantly related plant species (Gilbert et al. 2015, Wandrag et al. 2020). In line with expected differences between conspecific and heterospecific PSFs, this relationship between phylogenetic distance and PSF outcomes should especially be strong in low-diversity plant communities, where specialist plant antagonists are expected to be abundant.

Here, we established a three-phase greenhouse experiment to examine how soil legacies of different plant diversity levels may affect patterns of conspecific and heterospecific PSFs among eight common grassland plant species. To this aim, we first established a ‘pre-conditioning’ phase, in which we conditioned soils with plant communities of different species diversity (one, two, four or eight species) and then created soil mixtures that contained soil conditioned by each of the eight species, but that differed in the level of plant diversity at which these soils were conditioned. We used these mixtures of soils conditioned by each of the eight species as a starting point to establish a regular PSF experiment with two experimental phases. We expected that soil mixtures consisting of soils conditioned by species monocultures contained relatively high abundances of specialist plant antagonists, while plant-antagonist communities in soil mixtures containing soils conditioned by eight-species mixtures were assumed to be dominated by generalists (Fig. 1A). As such, we tested the hypothesis that compared to heterospecific PSFs, conspecific PSFs are most negative in soil mixtures with a legacy of the lowest plant diversity, but become less

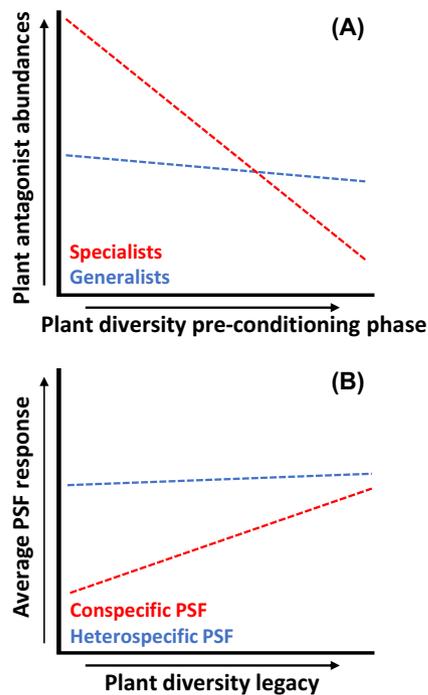


Figure 1. Hypothesized relationships between plant diversity and the accumulation of belowground specialist and generalist plant antagonists in the pre-conditioning phase (A) and between plant diversity legacy and subsequent average plant–soil feedback (PSF) responses to conditioning by conspecific and heterospecific plants (B).

negative in soil mixtures with legacies of increasing plant diversity (Hypothesis 1, Fig. 1B). Additionally, we tested the hypothesis that the predictability of pairwise PSFs by phylogenetic distance between conditioning and responding species decreases with an increasing plant diversity legacy (Hypothesis 2).

Material and methods

Plant species and seed germination

We tested our hypotheses using eight perennial grassland species commonly found in southern-German grasslands: *Plantago lanceolata* (Plantaginaceae), *Prunella vulgaris* (Lamiaceae), *Trifolium pratense* (Fabaceae), *Cerastium fontanum*, *Silene flos-cuculi* (Caryophyllaceae), *Hypericum perforatum* (Hypericaceae), *Leontodon hispidus* (Asteraceae) and *Poa pratensis* (Poaceae). Before each of the three experimental phases, seeds of all plants were germinated in trays containing regular potting soil 2–3 weeks before the start of the different experimental phases, and placed in a greenhouse compartment (16 h light/8 h dark; 19°C/18°C).

Experimental setup

Overview

Our experiment consisted of three subsequent phases (Fig. 2). In the first ('pre-conditioning') phase, we established

communities of different diversity levels (one, two, four or eight species), creating 40 communities for each diversity level, divided over five replicate blocks. In this way, we created soils with legacies of different diversity levels. To examine the consequences of community diversity on soil conditioning effects by single plants, we subsequently created block-level soil mixtures for each level of pre-conditioning phase diversity, with each of these mixtures containing soil from all eight species. Using shoot biomass data of the pre-conditioning phase, we also assessed the contributions of the individual species to the soil legacy effects present in these soil mixtures. We used these soil mixtures to perform a two-phase PSF experiment, by first conditioning all soil mixtures with monocultures of each of the eight plant species (conditioning phase) and, subsequently, testing the total biomass responses (combining shoot and root biomass) of all eight plant species in each soil from the conditioning phase.

Pre-conditioning phase

Prior to the start of the experiment, on 16 and 17 June 2021, we created a background soil by mixing 240 l of sieved field soil (15-mm sieve), collected from a species-rich grassland (in which most of the examined species occur (47°41'29"N, 09°10'45"E), with 120 l of sieved potting soil (5-mm sieve) and 450 l of a 1:1 sand–vermiculite mixture. Using this mixed starting soil, we subsequently filled 160 5-l pots. On 21 and 22 June 2021, we established the plant communities. First, we established five monoculture pots for each of the eight plant species, by planting eight individuals in a circular pattern in each of the pots, resulting in 40 monoculture pots in total. Pots with two-species (40 pots) and four-species mixtures (40 pots) were established by planting random combinations of two or four species, respectively, while making sure that each of the species was present equally often in each set of eight pots assigned to one experimental block. These species combinations also differed between experimental blocks, although some species combinations occurred in multiple experimental blocks. Finally, 40 eight-species mixture pots were established by planting each of the pots with single individuals of all eight species. In two-species mixture pots, species' seedlings were planted in an alternating pattern, while in four- and eight-species mixture pots seedlings were planted in a randomized order to reduce the possibility of confounding effects caused by specific neighboring plant combinations. All 160 pots were then placed in individual saucers on a large table in a greenhouse compartment and arranged according to a randomized block design with five experimental blocks. Pots were regularly watered, and each pot received 300 ml of 1‰ Everis Scotts Universol Blue fertilizer solution (18 – 11 – 18 + 2.5 MgO + TE, ICL Deutschland Vertriebs GmbH) on 26 August. On 11 October, after 16 weeks of plant growth, we separately harvested shoots for each of the plant species and dried them at 70°C until constant weight. Then, on 12 and 13 October, we collected soil from each pot by separating it from the root systems, which we washed and dried at 70°C. As the root systems of the different plants in each pot could not be separated, we only obtained pot-level root biomass

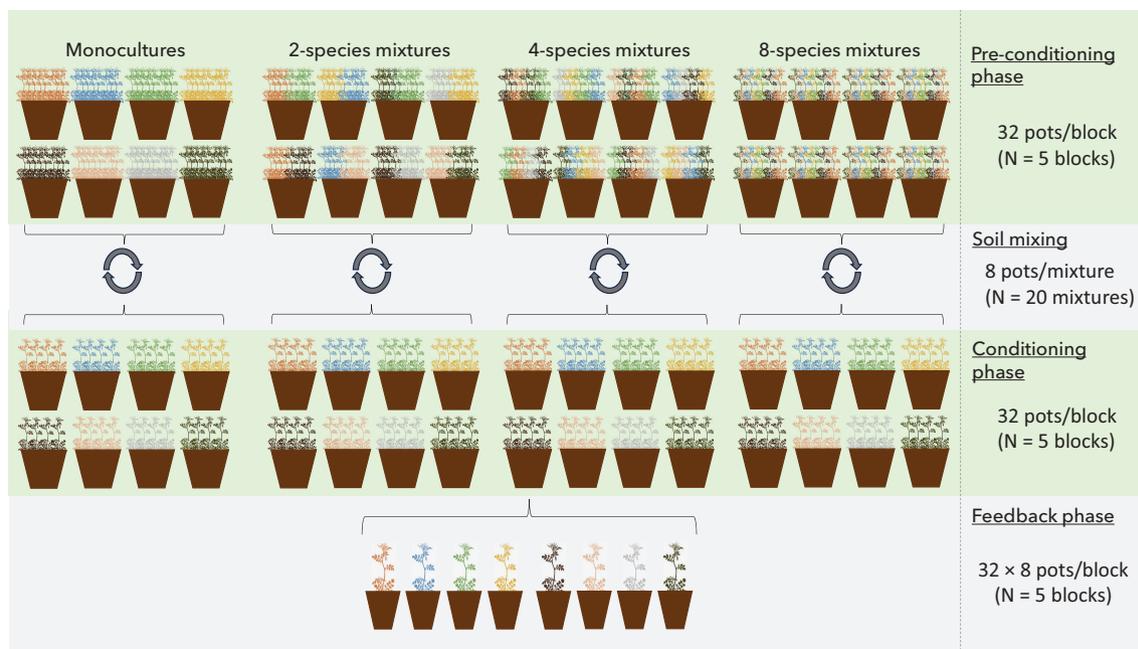


Figure 2. Schematic overview of our three-phase greenhouse experiment. In the first (pre-conditioning) phase (16 weeks), we established 5-l pots with eight plant individuals, of either one, two, four or eight different plant species. Sets of eight pots, combinedly containing the same number of plant individuals per species, were then used to create soil mixtures that each contained soil conditioned by each of the species. These soil mixtures were then used to establish eight new 5-l pots, which we planted with monocultures (four individuals) of each species to start the conditioning phase. After 12 weeks of plant growth, we divided soil from each of the conditioning-phase pots over eight small pots, planted with single individuals of each of the eight plant species. After 7.5 weeks of plant growth, the experiment was harvested to determine diversity legacy effects on plant performance, and their dependency on subsequent conspecific or heterospecific soil conditioning.

measurements. Collected soils were then used to set up the conditioning phase.

Conditioning phase

Prior to the start of the conditioning phase, we filled the bottom layers of 160 5-l pots with two liters of background soil, consisting of 13% sieved (5 mm) and autoclaved (130°C) potting soil and 87% sand–vermiculite mixture. Then, for each of the five experimental blocks in the pre-conditioning phase, we mixed two liters from each of the eight pots of the same plant diversity level, resulting in five 16-l mixtures for each of the different diversity treatments, containing soil conditioned by 64 plant individuals ($n = 8$ per plant species). These mixtures in principle thus contained equal amounts of soil conditioned by the eight different plant species, but differed in the level of surrounding plant diversity at which these species were conditioning the soil. Then, on 14 October 2021, we used each soil mixture to fill the upper half of eight conditioning-phase pots, and planted each pot with four seedlings of one of the eight plant species, after which the pots were placed in a greenhouse compartment and arranged according to a randomized block design with the same five replicate blocks (i.e. pots with soil mixtures from block 1 in the pre-conditioning phase were also placed in block 1 in the conditioning phase). After two and nine weeks of plant growth, each pot respectively received 300 and 100 ml of fertilizer solution. On 10 January 2022, after 12 weeks of

growth, we clipped all shoots and dried them at 70°C, after which we separately collected soils and roots from each pot. Roots were then washed and dried at 70°C. Soils were stored cool (10°C) until the start of the feedback phase.

Feedback phase

From 12 January until 14 January 2022, we used soil from each of the conditioning-phase pots to fill eight 500 ml pots with 400 g of fully conditioned soil, resulting in 1280 pots. We then planted these pots with single seedlings of one of the eight plant species, and placed them in a greenhouse compartment according to a randomized block design with the same five replicate blocks as used for the preceding phases. Directly after planting, we counted the number of leaves of each of the seedlings, which we later used as a proxy for initial plant size. The pots were regularly watered and received 50 ml of fertilizer solution after ± 18 days of plant growth. After 7.5 weeks of plant growth, we clipped all shoots, collected and washed root systems, and dried roots and shoots at 70°C.

Statistical analysis

We performed all statistical analyses in R ver. 4.2.1 (www.r-project.org).

Pre-conditioning phase

To examine whether changes in plant diversity were associated with changes in community biomass, we modelled

natural-log-transformed total first-phase biomass using a general linear mixed effects model, with species combination and block as random intercepts and plant diversity as numerical explanatory variable. To determine whether contributions of individual species to community-level biomass may have changed with increasing plant diversity, we modelled average plant individual shoot biomass in response to the fixed explanatory variables plant diversity and species identity, as well as their interaction term, including species combination and replicate block as random intercepts in the model.

Conditioning phase

To examine whether monoculture biomass in the conditioning phase was influenced by the diversity of plant communities in the pre-conditioning phase, we modelled natural-log-transformed monoculture biomass in response to the explanatory variable 'diversity legacy', with 'conditioning species identity', 'soil mixture' and 'block' as random intercepts. To control for potential variation in nutrient availability caused by differences in overall biomass accumulation in pre-conditioned soils, for example, we added soil-mixture-level 'total pre-conditioning phase plant biomass' as a covariate in our model. Moreover, because monoculture biomass in the conditioning phase may potentially be affected by soil legacy effects associated with the biomass of the respective species in the pre-conditioning phase, we also added 'pre-conditioning phase conspecific biomass' as a covariate in this model. To additionally examine whether monoculture biomass in the conditioning phase was affected by the aforementioned treatments in a conditioning-species-specific manner, we also constructed a similar model with 'conditioning species identity', as well as its interactions with 'diversity legacy' and 'pre-conditioning phase conspecific biomass' as fixed effects. In both models, we allowed heterogeneous variances for 'conditioning species' using the *varIdent* function ('nlme' package, Pinheiro et al. 2014).

Feedback phase

To assess whether plants grew differently in soil conditioned by conspecific individuals than in soil conditioned by heterospecific individuals, and whether these differences depended on the legacy effect of pre-conditioning-phase plant diversity, we modelled natural-log-transformed plant total biomass in the feedback phase in response to the fixed explanatory variables 'diversity legacy' and 'feedback type' (conspecific versus heterospecific). In this model, 'block', 'conditioning phase pot identity', 'pre-conditioning phase soil mixture', 'conditioning species identity' and 'responding species identity', as well as its two-way and three-way interactions with 'diversity legacy' and 'feedback type' were included as random intercepts, whereas 'initial leaf number', 'conditioning phase plant biomass' and natural-log-transformed 'pre-conditioning phase conspecific biomass' were modelled as covariates. To examine whether feedback effects on conspecific individuals were stronger when respective species' biomass in the pre-conditioning phase was higher, we also added the interaction between 'feedback type' and 'pre-conditioning phase

conspecific biomass' to the model. To subsequently examine whether diversity legacy effects on conspecific and heterospecific feedbacks may depend on the identity of the responding species, we constructed a similar general linear mixed effects model, which also included 'responding species identity' as a fixed factor, as well as its two-way and three-way interactions with 'diversity legacy', 'feedback type' and 'pre-conditioning phase conspecific biomass'. We then used post hoc Tukey tests (Lenth et al. 2018) to examine which species exhibited significant differences in total biomass between conspecific-conditioned and heterospecific-conditioned soils. Moreover, to test whether legacy effects of diversity and pre-conditioning phase biomass significantly affected species' biomass in the feedback phase, we constructed similar general linear mixed effects models for each individual species.

Finally, we examined whether differences in feedback response between soils conditioned by conspecific and heterospecific individuals could be explained by the phylogenetic distance between conditioning and responding species. To this aim, we first calculated block-level pairwise PSF values for each pair of conditioning-responding species as done by Wandrag et al. (2020), using the formula: pairwise PSF = $\ln(\text{biomass species } A_{\text{conspecific}} / \text{biomass species } B_{\text{heterospecific}}) - \ln(\text{biomass species } A_{\text{heterospecific}} / \text{biomass species } B_{\text{conspecific}})$, in which conspecific and heterospecific respectively indicate soils conditioned by conspecific and heterospecific monocultures. Positive pairwise PSF values indicate that plants grew better in soils conditioned by conspecific monocultures than in soils conditioned by heterospecific monocultures. We then extracted phylogenetic distances from the phylogenetic tree used in van Kleunen et al. (2020), which was based on the mega-phylogeny by Smith and Brown (2018). Using these data, we first tested whether phylogenetic distance predicted average PSF outcomes, and whether this depended on the diversity legacy of the soil. To this aim, we constructed a general linear mixed effects model with average pairwise PSF as response variable and phylogenetic distance and diversity legacy, as well as their interaction, as fixed explanatory variables, and with 'plant pair' as random intercept. To also test whether PSF outcomes became more variable with increasing phylogenetic distance, and whether this depended on diversity legacy, we constructed a similar model as the previous one, but now modelled the (square-root-transformed) absolute values of the residuals of the former model as response variable.

Results

Pre-conditioning phase

First-phase total community biomass was not affected by plant diversity (Supporting information). Individual species showed strongly species-specific biomass responses to increasing plant diversity (Species identity \times Plant diversity: $\chi^2 = 30.42$, $p < 0.001$, Supporting information), with average shoot biomass of *Cerastium fontanum*, *Plantago lanceolata*, *Silene flos-cuculi* and *Trifolium pratense* individuals increasing

with increasing plant diversity and average shoot biomass of *Hypericum perforatum*, *Leontodon hispidus*, *Poa pratensis* and *Prunella vulgaris* individuals decreasing with increasing plant diversity (Supporting information).

Conditioning phase

Total biomass of conditioning-phase monocultures was higher in soil mixtures with legacies of more productive pre-conditioning-phase communities (total plant biomass pre-conditioning phase: $\chi^2 = 5.23$, $p = 0.02$), but was not affected by plant diversity of the pre-conditioning phase (Supporting information). Overall, monoculture biomass was not affected by the biomass of the respective species in the pre-conditioning phase ($\chi^2 = 0.09$, $p = 0.76$, Supporting information). Monoculture biomass strongly varied among species ($\chi^2 = 233.27$, $p < 0.001$), but this among-species variation in monoculture biomass did not depend on plant-diversity legacy ($\chi^2 = 2.95$, $p = 0.89$) or pre-conditioning-phase plant biomass ($\chi^2 = 7.54$, $p = 0.37$, Supporting information).

Feedback phase

On average, plants accumulated more biomass when growing in soils conditioned by heterospecific individuals than in soil conditioned by conspecific individuals (Fig. 3, Supporting information), and did not show differences in biomass accumulation among soils with different legacies of plant diversity. However, a subsequent analysis of species-specific responses revealed that total biomass in the feedback phase depended on the legacy of plant diversity, but in species-specific ways (Table 1). In particular, individuals of *Cerastium fontanum* and *Plantago lanceolata*, and to a lesser extent individuals of *Hypericum perforatum*, accumulated most biomass in soils with a legacy of the highest plant diversity, whereas *Trifolium pratense* individuals appeared to accumulate less biomass

in soils with a legacy of the highest plant diversity (Fig. 4), although the latter effect was not significant. Biomass accumulation of the other species appeared to be unaffected by plant-diversity legacy (Fig. 4).

Species' biomass responses in the feedback phase in part were also explained by biomass averages of the respective species in the soil mixtures of the pre-conditioning-phase, and this effect did not depend on whether the soil mixtures were subsequently conditioned by conspecific or heterospecific individuals (Table 1). *Prunella vulgaris* and *Silene flos-cuculi* individuals appeared to accumulate more biomass in soil mixtures with a legacy of high conspecific plant biomass than in soil mixtures with a legacy of low conspecific plant biomass (Fig. 5). In contrast, *Cerastium fontanum* and *Poa pratensis* individuals in particular accumulated less biomass in soil mixtures with a legacy of high conspecific plant biomass than in soil mixtures with a legacy of low conspecific plant biomass (Fig. 5).

Finally, plant species differed in their responses to soils conditioned by conspecific and heterospecific species (Fig. 3), but this effect did not depend on diversity legacy (Table 1). *Prunella vulgaris* tended to perform better in soils conditioned by conspecific individuals than in soil conditioned by heterospecific individuals (post hoc analysis: $p = 0.089$), whereas most other plant species tended to perform better in heterospecific- than in conspecific-conditioned soils (Fig. 3). However, post hoc analysis revealed that only *Plantago lanceolata* biomass was significantly lower in conspecific-conditioned than in heterospecific-conditioned soil ($p < 0.001$) (Fig. 3).

Overall, pairwise PSF outcomes were not affected by plant diversity legacy and were not explained by pairwise phylogenetic distance (Supporting information). Pairwise PSF outcomes also did not become more variable with phylogenetic

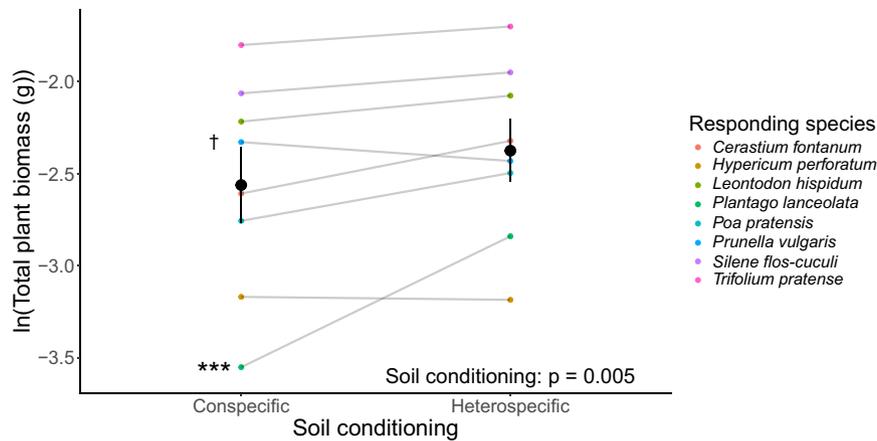


Figure 3. Biomass responses to soil conditioned by conspecific or heterospecific plants, averaged over the different diversity legacy levels. Black dots and whiskers represent mean total biomass values \pm standard errors, calculated based on species' average biomass responses ($n = 8$), which are indicated with colored dots. Linear mixed effects modelling of overall treatment effects on plant biomass indicated a significant difference in plant biomass between conspecific-conditioned and heterospecific-conditioned soils. Subsequent linear mixed effects modelling of species' responses, followed by post hoc analysis, indicated that two species showed marginally significantly (*Prunella vulgaris*) or significantly different (*Plantago lanceolata*) biomass responses to conspecific-conditioned and heterospecific-conditioned soils, indicated at the left side of the panel. *** $p < 0.001$, † $0.05 < p < 0.1$.

Table 1. Effects of diversity legacy, conspecific biomass in the pre-conditioning phase, plant–soil feedback type (conspecific versus heterospecific), responding plant species and their interactions on plant biomass in the feedback phase. Significant results ($p < 0.05$), based on type-II Wald χ^2 tests, are shown in bold.

Fixed effects	χ^2	df	p
Leaf number	200.057	1	< 0.001
ln(Biomass phase 2)	0.528	1	0.47
ln(Conspecific biomass phase 1) (CB)	0.193	1	0.66
Diversity legacy (L)	0.964	1	0.33
Feedback type (F)	13.209	1	< 0.001
Responding species (RS)	617.264	7	< 0.001
CB \times F	1.040	1	0.31
CB \times RS	19.751	7	0.01
L \times F	0.815	1	0.37
L \times RS	19.600	7	0.01
F \times RS	18.932	7	0.01
CB \times F \times RS	3.281	7	0.86
L \times F \times RS	9.151	7	0.24
Random effects	SD		
Block	0.028		
Pot ID Phase 2	0.152		
Species ID Phase 2	0.080		
Soil mixture phase 1	0.072		

distance ($\chi^2 = 0.0094$, $p = 0.92$), and PSF outcome variability was not significantly affected by diversity legacy ($\chi^2 = 2.64$, $p = 0.10$), or the interactive effect of diversity legacy and phylogenetic distance ($\chi^2 = 1.79$, $p = 0.18$) (Supporting information).

Discussion

Our results show that soil legacy effects of plant diversity can affect future plant performance, and that these effects can be positive or negative, depending on the responding plant species. Previous research already showed the importance of plant diversity effects on subsequent plant–soil interactions by creating mixtures using monoculture soils from different numbers of plant species (Hendriks et al. 2013, Zhang et al. 2020). However, our results show that plant-diversity effects on plant–soil interactions also act through plant-community-mediated changes in soil-conditioning effects of individual species. Hence, our results further highlight the importance of neighboring plant communities for the interactions of single plants with soil biota (Mommer et al. 2018, Ampt et al. 2022, Chen and van Kleunen 2022). Yet, the observed species-specific responses to these legacy effects of conditioning species' diversity do not provide support for the expected overall weakening of negative PSFs with increasing plant diversity (Thakur et al. 2021). The diversity legacy effects on plant performance were detected in the feedback phase of our experiment, and thus persisted throughout the experimental phase in which soil mixtures were conditioned by monocultures of each of the plant species. Our study therefore provides further evidence that plant communities can leave long-lasting

soil legacy effects (Wubs et al. 2019, Hannula et al. 2021) and adds to these studies that such legacy effects depend on plant community diversity.

We expected that plants would most suffer from negative PSFs in conspecific-conditioned soils with a legacy of the lowest plant diversity and that legacy effects of plant diversity would weaken differences between conspecific and heterospecific PSF outcomes (hypothesis 1), due to the disappearance, or strongly decreasing abundances, of specialist plant antagonists with increasing plant diversity (Mommer et al. 2018). Our results, however, did not indicate that differences between conspecific and heterospecific PSFs depended on diversity legacy effects. Instead, independent of diversity legacy, plants on average grew more poorly in soil conditioned by conspecifics than in soil conditioned by heterospecifics, thus indicating consistently negative effects of belowground specialist plant antagonists. Our analyses of species-specific conspecific and heterospecific feedback responses also indicated that most, but not all, species on average grew better in soil conditioned by heterospecifics than in soil conditioned by conspecifics. Yet, this difference was only significant for *Plantago lanceolata*, which also was the only species that consistently grew more poorly in its own soil than in soils of other plant species (Fig. 3), indicating an overall limited role of 'true' host specialists in this study (Semchenko et al. 2022, Wilschut et al. 2023). While the impact of such host specialists may have been stronger with a longer pre-conditioning and conditioning phase, we note that these phases are longer than many PSF studies that detected significant differences between conspecific and heterospecific feedbacks (for example, van de Voorde et al. 2011). Combined, these results either suggest that individual plants in the first phase did not accumulate lower (relative) abundances of specialist plant antagonists when growing in a more diverse community than when growing in a less diverse community. Alternatively, in case the rapid accumulation of specialist plant antagonists during the monoculture conditioning phase obscured differences in their actual abundances at the end of the pre-conditioning phase, these results at the very least suggest that specialist plant antagonists did not completely 'disappear' from the community in response to increasing plant community complexity (Mommer et al. 2018). The positive diversity legacy effects on the performance of some of the plant species may nevertheless still be explained by overall reductions in plant antagonist abundances (Mitchell et al. 2002, Schnitzer et al. 2011) or diversity (Mommer et al. 2018, Semchenko et al. 2018), which may be driven by positive plant diversity effects on disease-suppressive soil biota (Latz et al. 2012).

In line with the analysis of patterns of conspecific and heterospecific PSFs, we did not find evidence that diversity legacy altered pairwise PSF patterns or its relationship with phylogenetic distance (hypothesis 2). Firstly, pairwise PSFs were not more negative in low diversity-legacy soil than in high diversity-legacy soil, which would be expected in case species exhibited more strongly negative conspecific feedbacks in low diversity-legacy soils. Secondly, when specialist plant antagonist abundances decreased with increasing diversity legacy,

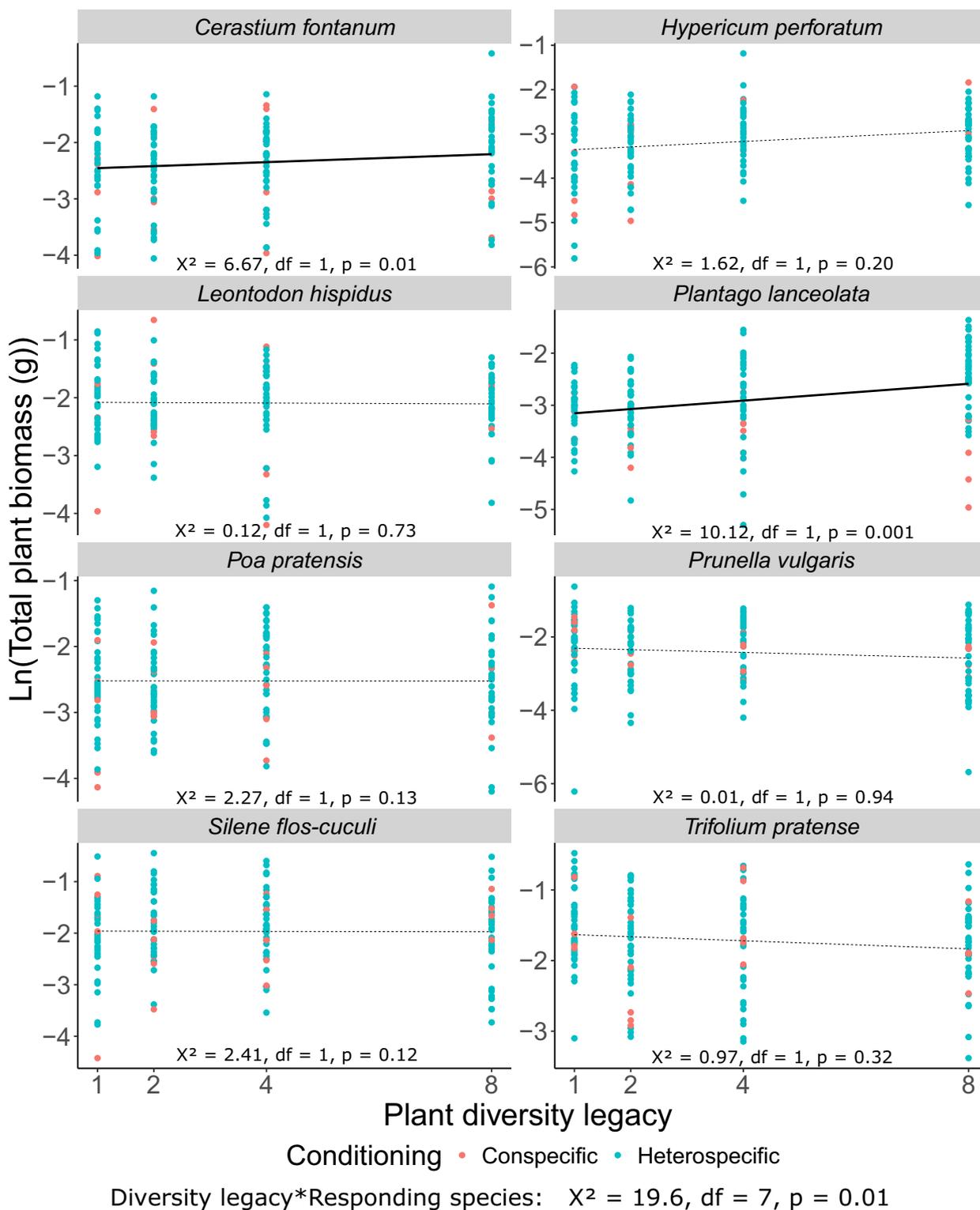


Figure 4. Species-specific relationships between plant diversity legacy and total biomass in the feedback phase. Dots represent species biomass responses to soils conditioned by heterospecific (blue) or conspecific plant monocultures (red). Within-panel statistical information indicates the species-level tests of diversity legacy effects on plant biomass in the feedback phase, while statistical information below the figure indicates the visualized statistical interaction. Solid, dashed and thin dashed lines represent slopes of simple linear regression models between diversity legacy and total biomass of individual plants for, respectively, significant, marginally significant and non-significant diversity legacy effects. Note that diversity legacy refers to the number of plant species simultaneously conditioning the soil in the pre-conditioning phase, and that each pre-conditioning phase soil mixture comprises soil from all eight plant species.

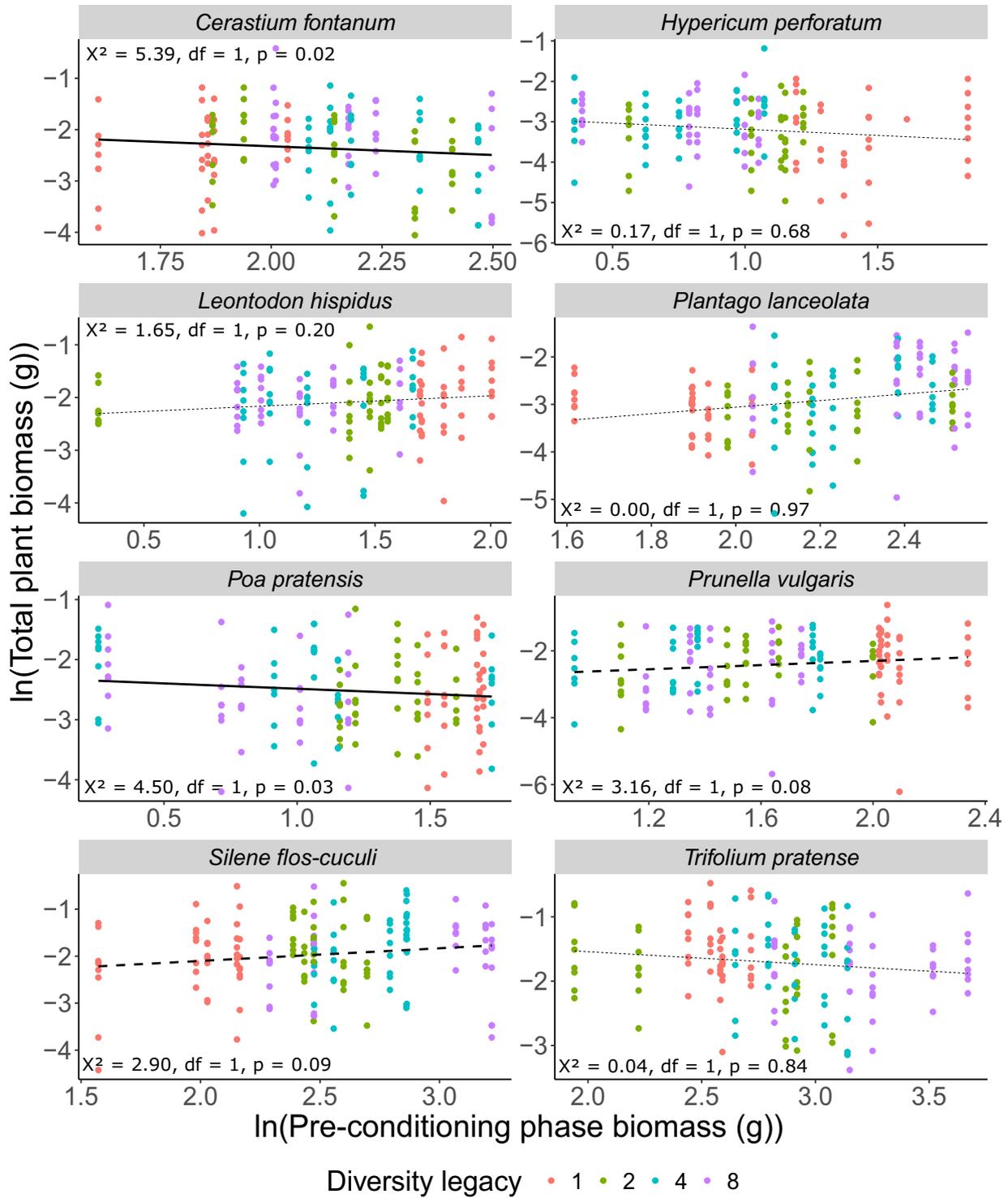


Figure 5. Species-specific relationships between average species biomass in soil mixtures ($n=5$ per diversity treatment) from the first (pre-conditioning) phase and conspecific biomass in the feedback phase. Within-panel statistical information indicates the species-level tests of pre-conditioning phase biomass effects on plant biomass in the feedback phase, while statistical information below the figure indicates the visualized statistical interaction. Solid, dashed and thin dashed lines represent slopes of simple linear regression models between natural-log transformed conspecific biomass in the first ('pre-conditioning') phase and total biomass of individual plants for, respectively, significant, marginally significant and non-significant diversity legacy effects.

the predictability of pairwise PSFs by phylogenetic distance would also be expected to decrease with increasing diversity legacy, as more closely related species should share a higher proportion of specialist plant antagonists (Gilbert and Webb 2007, Gilbert and Parker 2016). Although the limited number of pairwise PSF observations in our analysis may limit the detectability of such an effect, we did not observe any indication of an effect of diversity legacy on the relationship between phylogenetic distance and pairwise PSF (Supporting information). Moreover, we did not find evidence that the variation in pairwise PSF outcomes increased with increasing phylogenetic distance, contrasting to what was found in the meta-analysis of Wandrag et al. (2020). However, the limited number of pairwise comparisons of closely related (i.e. con-familial) species hampers the drawing of strong conclusions on this relationship. As such, future studies aimed at examining the predictability of heterospecific feedbacks by phylogenetic distance should select a more balanced set of closely and distantly related species.

We set up our experiment to examine whether plant-diversity effects on PSFs are mediated by changes in soil-conditioning effects of single plant individuals, caused by the diversity of the community these single plants grew in. Yet, manipulations of community diversity also altered competitive hierarchies in the examined plant communities, causing species-specific changes in average biomass of individual plants in the pre-conditioning phase (Supporting information). Consequently, changing species' contributions to soil conditioning effects by plant communities may have partly altered the anticipated diversity legacy effects. For example, potential reductions of host detectability by specialist plant antagonists, which are expected with increasing host diversity (Mommer et al. 2018), may have been mitigated in the case of *Cerastium fontanum* and *Plantago lanceolata*, which grew better with increasing community diversity. In contrast, such reductions in host detectability could have been amplified for the other plant species, which grew most poorly in high-diversity communities (Parker et al. 2015). Yet, associated changes in conspecific PSFs that would be expected with such profound reductions in host abundance were not observed for any of the latter species. Yet, species biomass in the first phase was negatively or positively associated with conspecific biomass responses in the feedback phase, suggesting biomass-dependent accumulation of beneficial and antagonistic soil biota.

Previous studies have shown that soil legacy effects of single plant individuals were species-specifically altered by subsequently establishing plant species, leading to altered PSF patterns (Wubs and Bezemer 2018, Kuřáková et al. 2023). It was also shown that repeated conditioning by conspecific individuals intensified negative PSFs (Wubs and Bezemer 2018). In contrast, conspecific feedback effects in our experiment were not stronger in soils with a history of high conspecific biomass, indicating that legacy effects established in the pre-conditioning phase were too strong to be significantly modified by subsequent conditioning by monocultures, although the latter phase also lasted for a shorter period than the pre-conditioning phase. We speculate that soil conditioning effects

of individual species may have been strongly buffered by the likely complex and diverse soil communities (Schmid et al. 2021, Zhang et al. 2022), associated with legacy effects of multiple species, but this needs further testing. Previous research showed that soil legacy effects of plant growth may fade over time, but persist when plant litter remains in the soil (He et al. 2023). Our research adds to this knowledge by suggesting that soil legacy effects may also to some extent be preserved by continued plant growth, independent of the identity of subsequently establishing plant species.

In conclusion, our study shows that soil conditioning effects of plant individuals depend on plant community diversity, and that such diversity legacy effects can positively or negatively affect future plant performance. However, we did not find evidence for diversity-driven changes in patterns of conspecific- and heterospecific PSFs, indicating that host detectability by, and the relative accumulation of, specialist plant antagonists were not modified by community diversity. At the same time, our results also indicate that while plant species on average grow more poorly in soil conditioned by conspecifics than in soils conditioned by heterospecifics, they rarely exhibit the poorest performance in their own soil, indicating a limited role for true host-specialist plant antagonists as main drivers of PSFs (Semchenko et al. 2022). Our study further shows that the soil legacy of diverse plant communities may be long-lasting and not quickly modified by subsequently establishing plant monocultures. This is relevant information for agricultural systems, in which the use of cover crop mixtures may help to establish beneficial legacy effects on subsequent crop growth (Barel et al. 2018). Our results suggest that such legacy effects may persist beyond the first period of subsequent plant growth, although future studies should elucidate their temporal dynamics over longer time periods and in field settings.

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Author contributions

Rutger A. Wilschut: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Mark van Kleunen:** Conceptualization (supporting); Formal analysis (supporting); Visualization (supporting); Writing – review and editing (equal).

Data availability statement

Data are available from Figshare: <https://doi.org/10.6084/m9.figshare.29572595.v1> (Wilschut and van Kleunen 2025).

Supporting information

The Supporting information associated with this article is available with the online version.

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